09/781,796

09/29/03 RCG

Amendments to the claims:

Please cancel claims 1, 2, 4-13 and 15-18 without prejudice or disclaimer. Applicants reserve the right to prosecute the subject matter thereof in future applications.

Claims 1 – 2 (cancelled)

Claim ~~3.~~ 1 (currently amended) An isolated and purified ATP diphosphohydrolase obtainable from ~~pig pancreatic zymogen granules~~ a mammalian tissue characterized by the following physico-chemical properties:

- a catalytic unit of a molecular weight on denaturing polyacrylamide gel electrophoresis of about 54 KDa ~~in its native form~~;
- a deglycosylated form of said catalytic unit of a molecular weight on SDS-PAGE of about 35 KDa; and
- characterized in that it comprises the amino acid sequence defined in SEQ. ID. NO: 7.

Claims 4-13. (cancelled)

Claim ~~14.~~ 2 (currently amended) A method for reducing platelet aggregation and thrombogenicity in a human or nonhuman animal comprising the step of ~~increasing the activity of~~ treating with an effective amount of the ATP disphosphohydrolase of claim [[1]] 1 sufficiently to reduce platelet aggregation and thrombegenicity in the human or nonhuman animal.

Claims 15-18 (cancelled)

Claim ~~19.~~ 3 (new) A process for purifying an ATP-diphosphohydrolase enzyme from a tissue capable to convert ATP to ADP and ADP to AMP which comprises:

a) obtaining a subcellular microsomal fraction from an homogenate of said tissue;

b) solubilizing said microsomal fraction in the presence of a non-ionic detergent;

c) centrifuging said solubilized microsomal fraction to obtain a supernatant containing said enzyme;

d) submitting said supernatant to an ion-exchange chromatography to obtain a first enzyme eluate;

e) submitting said first eluate to an affinity column chromatography to obtain a second enzyme eluate; and

f) submitting said second eluate to a separation step on a non-denaturing gel electrophoresis to recover said enzyme free of any contaminant, the presence of said contaminant being monitored by overstaining said gel in a silver nitrate dye or Coomassie Blue dye,

whereby an isolated and purified ATP diphosphohydrolase according to claim 1 is obtained.

Claim 4. (new) A process according to claim 3 wherein said ion exchange chromatography is achieved on a column containing Diethylaminoethyl (DEAE).

Claim 5. (new) A process according to claim 4 wherein said column is a DEAE agarose column.

Claim 6. (new) A process according to claim 3 wherein an aliquot of said enzyme is further submitted after step f) to a polyacrylamide gel electrophoresis under denaturing conditions to verify its homogeneity and to obtain its apparent molecular weight.

Claim 7. (new) A process according to claim 3 wherein said enzyme is obtained from pig pancreatic zymogen granules and has an apparent molecular weight of about 54 Kilodaltons.

Claim 8. (new) A process according to claim 7 wherein, between steps e) and f), a step of deglycosylation is included, and whereby the apparent molecular weight is shifted from 54 to 35 KDa.

Claim 9. (new) A method for reducing platelet aggregation and thrombogenicity comprising an administration of the ATP diphosphohydrolase of claim 1.

Claim 26. (new)A method for reducing platelet aggregation and thrombogenicity comprising an administration of an ATP diphosphohydrolase comprising the amino acid sequence defined in SEQ ID NO:7.

Claim 27. (new) A composition for reducing platelet aggregation and thrombogenicity which comprises as an active ingredient the ATP diphosphohydrolase of claim 3, together with an acceptable pharmaceutical carrier.

Claim 28. (new) An aggregation and thrombogenicity-reducing composition, which comprises as an active ingredient the mammalian ATP diphosphohydrolase of claim 3, together with a pharmaceutically acceptable carrier.

Claim 29. (new) A composition for converting ATP into ADP and/or ADP into AMP, which comprises as an active ingredient the mammalian ATP diphosphohydrolase of claim 3, together with a pharmaceutically acceptable carrier.

Claim 30. (new) A process for purifying an ATP diphosphohydrolase enzyme which can convert ATP to ADP and/or ADP to AMP, said process comprising:

a) separating a crude fraction of said enzyme from contaminating material by centrifugation;

b) submitting said enzyme of a) to at least one of ion-exchange chromatography and affinity column chromatography to obtain a purified enzyme eluate;

whereby an isolated and purified ATP diphosphohydrolase according to claim 3 is obtained.

Claim 31. (new) The process of claim 30, wherein said crude fraction is incubated with a non-ionic detergent, prior to centrifugation.

Claim 32. (new) The process of claim 31, wherein said enzyme of a) is submitted to at least one round of ion-exchange chromatography to yield a first enzyme eluate, and said first enzyme eluate is submitted to at least one round of affinity chromatography, to yield a second enzyme eluate.

Claim 33. (new) The process of claim 32, wherein said second enzyme eluate is electrophoresed on a non-denaturing gel, thereby recovering substantially pure ATP

diphosphohydrolase, and wherein a presence of contaminants in said substantially pure ATP diphosphohydrolase can be monitored by overstaining said non-denaturing gel in a silver nitrate dye or Coomassie Blue dye.

Claim ~~34~~ 16. (new) The process of claim ~~33~~ 17, wherein said ion exchange chromatography is achieved on a Diethylaminoethyl (DEAE) column.

Claim ~~35~~ 19. (new) The process of claim ~~34~~ 18, wherein said column is a DEAE agarose column.

Claim ~~36~~ 20. (new) The process of claim ~~30~~ 14, wherein said enzyme is obtained from a mammalian membrane preparation and has an apparent molecular weight of about 54 Kilodaltons.

Claim ~~37~~ 21. (new) A substantially pure mammalian ATP diphosphohydrolase characterized by the following physico-chemical properties:

- a catalytic unit of a molecular weight on denaturing polyacrylamide gel electrophoresis of about 54 KDa;
- a deglycosylated form of said catalytic unit of a molecular weight on SDS-PAGE of about 35 Kda.

Claim ~~38~~ 22. (new) A composition for use in the reduction of platelet aggregation and thrombogenicity comprising as an active ingredient the substantially pure mammalian ATP diphosphohydrolase of claim ~~37~~ 21, together with a pharmaceutically acceptable carrier.

Claim ~~39~~ 23. (new) A composition for converting ATP into ADP and/or ADP into AMP comprising as an active ingredient the substantially pure mammalian ATP diphosphohydrolase of claim ~~37~~ 21, together with a pharmaceutically acceptable carrier.